

AD

(Leave blank)

Award Number:
W81XWH-09-1-0106

TITLE:
Group II metabotropic glutamate receptors as potential pharmaceutical targets
for neurofibroma formation

PRINCIPAL INVESTIGATOR:
Michael Stern, Ph.D.

CONTRACTING ORGANIZATION:
William Marsh Rice University
Houston, TX 77005

REPORT DATE:
February 2010

TYPE OF REPORT:
annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

☒ Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 28-02-2010		2. REPORT TYPE annual		3. DATES COVERED 1 FEB 2009 - 31 JAN 2010	
4. TITLE AND SUBTITLE Group II metabotropic glutamate receptors as potential pharmaceutical targets for neurofibroma formation				5a. CONTRACT NUMBER W81XWH-09-1-0106	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Michael Stern, Ph.D. Email: stern@rice.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) William Marsh Rice University Houston, TX 77005				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Several lines of evidence suggest that neurofibroma formation involves hyperactivation of PI3K within the glial layer that surrounds the motor neurons, and yet the signals within glioblastoma which PI3K activity is regulated remain incompletely understood. Following our recent discovery that the Drosophila group II metabotropic glutamate receptor (DmGluRA) activates PI3K in motor neuron, we hypothesized that activation of DmGluRA might similarly activate PI3K in glia. In task #1, we proposed to test if inhibition of DmGluRA-PI3K activity in motor neurons is sufficient to activate PI3K in the analogue of the Schwann cell called the peripheral glia (as monitored by perineurial glial growth). We found that that inhibiting PI3K activity by introducing the DmGluRA112b null mutation or by expressing the PI3KDN transgenin in motor neurons did significantly increase perineurial glial growth. In task #2, we proposed to determine if DmGluRA activity in peripheral glia is required for PI3K activation. The stock construction required to address this task are almost complete and experiments are anticipated to begin within the next few weeks.					
15. SUBJECT TERMS molecular genetics; neuroscience; cell biology; cell signaling; model systems model systems genetics					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusions.....	6
References.....	6

INTRODUCTION

Several lines of evidence have suggested that neurofibroma formation occurs at least in part via hyperactivation of PI3K, which is a direct target of Ras activity (Dasgupta et al., 2005; Johannessen et al., 2005; Lavery et al., 2007). The involvement of PI3K in neurofibroma formation suggests that molecules that participate in the regulation of PI3K in peripheral nerves might be promising targets for therapeutic intervention. However, our understanding of the mechanisms by which PI3K activity in the nervous system is regulated is incomplete. Recently, my lab showed that in *Drosophila* larval motor neurons, PI3K is activated by the group II metabotropic glutamate receptor DmGluRA (Howlett et al., 2008), raising the possibility that antagonists of these group II receptors might prevent PI3K activation and thus act therapeutically in neurofibroma formation. In this exploratory-hypothesis development award, I proposed two tasks to extend these observations. First (task one), I proposed to determine if inhibition of the DmGluRA-PI3K pathway in motor neurons would be sufficient to increase growth of the outer, perineurial glial layer (analogous to the mammalian perineurium). Preliminary results suggest that this inhibition does indeed increase perineurial glial size. Second (task two), I proposed to determine if DmGluRA activity in peripheral glia (analogue of the Schwann cell) is required for increased perineurial glia. The fly stocks required to perform this task are almost finished, and the experiments to answer this question are expected to begin in the next few weeks.

BODY

Task one: Does the increased motor neuron excitability conferred by inhibiting the mGluRA-PI3K pathway promote perineurial glial growth?

In this aim, I proposed to inhibit DmGluRA-PI3K activity in motor neurons and, using electron microscopy, monitor the resulting effects on perineurial glial thickness. I proposed to perform these analyses both in a wildtype background, as well as in a background in which the *inebriated*-encoded neurotransmitter transporter was eliminated by chromosomal mutation (*ine*⁻). The use of the *ine*⁻ mutation was previously shown to sensitize the peripheral nerve to trophic effects of other mutations and to reveal effects on perineurial glial growth that were otherwise difficult to demonstrate.

We found that combining the *ine*⁻ mutation with genotypes that disrupt DmGluRA-PI3K activity in motor neurons does indeed significantly increase perineurial glial thickness. In particular, in *ine* mutants carrying the *DmGluRA* null mutation *DmGluRA*^{112b}, perineurial glial thickness was increased from 1.27 +/- 0.1 to 2.05 +/- 0.2 μ m (p=0.004, see Figure 1 below). In addition, in *ine* mutants in which PI3K activity in motor neurons was blocked by *D42-Gal4*-induced motor neuron expression of *PI3K*^{DN}, perineurial glial thickness was increased to 1.68 +/- 0.15 μ m. Although this effect is significant (p=0.048, see Figure 1 below), it is barely so and the increase in glial thickness is less than what we observe in *ine*; *DmGluRA*^{112b}. Why? We have recently obtained evidence from other experiments that the *PI3K*^{DN} transgene is a considerably weaker blocker of PI3K activity than the related transgene in which PTEN (phosphatase that antagonizes PI3K) is overexpressed. Therefore I hypothesize that the weak phenotype of

PI3K^{DN} results from a weak transgene. This possibility will be tested by constructing and analyzing larvae in which *PTEN* is overexpressed in an *ine* mutant background.

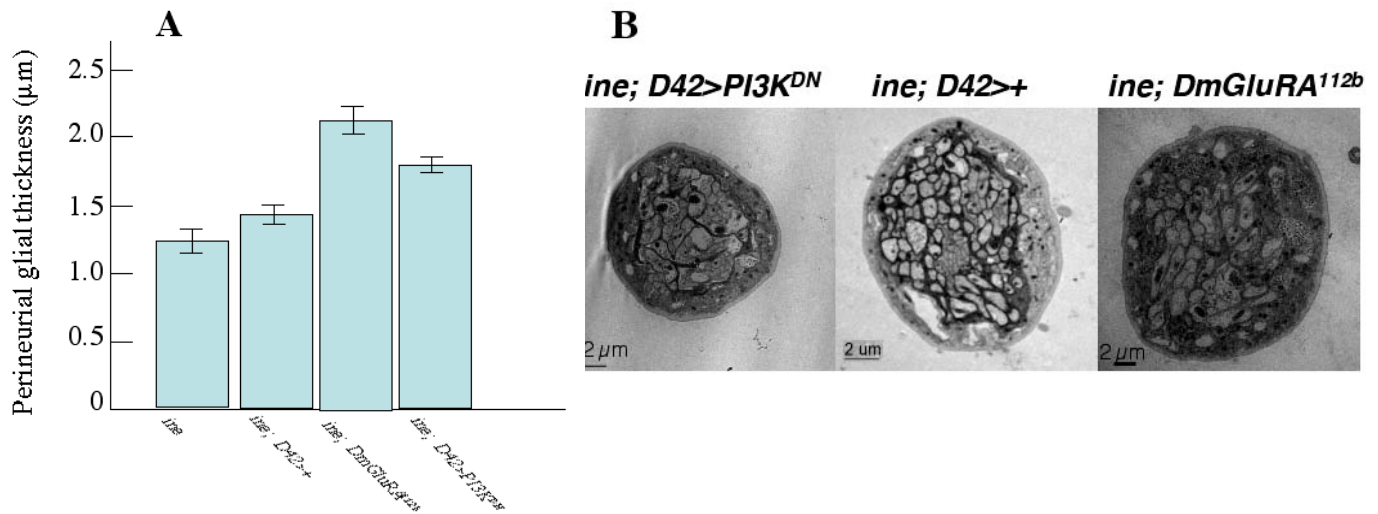


Figure 1: Perineurial glial thickness is increased in *ine; DmGluRA^{112b}* double mutants. A) Mean perineurial glial thickness (Y axis) +/- SEMs for the genotypes indicated along the X axis. The following genotypes showed significant differences in glial thickness (Student's t test): *ine* (lane #1, n=18) versus *ine; DmGluRA^{112b}* (lane 3, n=14), p=0.004. *ine; D42>+* (lane #2, n=31) versus *ine; D42>PI3K^{DN}* (lane #4, n=8), p=0.048. B) Representative transmission electron micrographs from larval peripheral nerves of the indicated genotypes. Scale bars as indicated.

In the next funding year, we will analyze the same larvae as above, except in an *ine*⁺ background. I predict that this wildtype background will restore perineurial glial growth to normal levels. We will also finish up measuring glial thickness in *ine* mutants overexpressing *Foxo*⁺. This experiment has been delayed because we picked up lethal mutations on the *ine; UAS-Foxo*⁺ chromosome but that problem has been solved and we are now ready to collect data.

Task two: Is mGluRA activity in peripheral glia required for the ability of motor neuron activity to promote perineurial glial growth? This question is based on previous observations from my lab that larvae doubly mutant for *ine* and second gene called *push*, which encodes an E3 ubiquitin ligase, also exhibit greatly increased perineurial glial thickness (Yager et al., 2001). I hypothesize that the increased perineurial glial thickness in *ine push* double mutants occurs because the *ine push* genotype increases glutamate release from motor nerve terminals, thus hyperactivating PI3K in the peripheral glia via DmGluRA. If so, then blocking DmGluRA specifically in peripheral glia is predicted to block this PI3K hyperactivation and thus decrease perineurial glial thickness in *ine push*.

To address this hypothesis, I proposed to construct *ine push* larvae in which peripheral glial DmGluRA activity was knocked down via RNAi. To accomplish this task, we constructed two stocks: in the first, we recombined the peripheral glial Gal4 driver *gli-Gal4* onto the *ine push* second chromosome, whereas in the second, flies carried *ine push* on the second chromosome, and *UAS-DmGluRA-RNAi* on the third chromosome. Because *push* mutations confer sterility, the second chromosomes of both stocks are balanced with the *CyO* balancer

marked with GFP (to enable us to distinguish homozygous from balanced larvae). We suffered from the usual problems of chromosomes picking lethal mutations, which prevents acquisition of homozygous larvae. This problem delayed construction of the necessary stocks but now we have the two stocks we need that will produce the desired larvae. We are now poised to cross together flies from the two stocks, and perform electron microscopy on the non-GFP labeled third instar larval progeny. In the next funding year, these larvae will be analyzed, and we will determine if DmGluRA expression specifically in peripheral glia is sufficient to rescue the glial growth phenotype of *ine push*; *DmGluRA*^{112b}.

KEY RESEARCH ACCOMPLISHMENTS

In preliminary results, we have found that blocking DmGluRA-PI3K activity in motor neurons in an *ine* mutant background increases perineurial glial thickness. However, this increased thickness is less extreme than the increased thickness observed in the *ine push* double mutant. These observations support the hypothesis originally put forth, but raise the possibility that regulators in addition to the neuronal DmGluRA-PI3K pathway play roles in the control of perineurial glial thickness.

REPORTABLE OUTCOMES

None.

CONCLUSIONS

The observations that blocking DmGluRA-PI3K activity in motor neurons in an *ine* mutant background increases perineurial glial thickness support the hypothesis originally put forth. However, the increase that we observe is much less than what we observed in the *ine push* double mutant, which was the phenotypic basis for the hypothesis. Therefore our preliminary conclusion is that DmGluRA-PI3K pathway inhibition does, indeed, increase perineurial glial thickness, but also that Push plays an additional role in regulating glial growth. Perhaps Push regulates perineurial glial growth by acting in the peripheral glia, as well as regulating perineurial glial growth by regulating excitability from the motor neuron. Resolving this issue is important but is beyond the scope of the current grant.

REFERENCES

Dasgupta B, Yi Y, Chen DY, Weber JD, Gutmann DH (2005) Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in neurofibromatosis 1-associated human and mouse brain tumors. *Cancer Res* 65:2755-2760.

Howlett E, Lin C-J, Lavery W, Stern, M (2008) A PI3K-mediated negative feedback regulates neuronal excitability, *PLoS Genetics* 4: e1000277.

Johannessen CM, Reczek EE, James MF, Brems H, Legius E, Cichowski K (2005) The NF1 tumor suppressor critically regulates TSC2 and mTOR. *Proc Natl Acad Sci USA* 102:8573-8578.

Lavery W, Hall V, Yager JC, Rottgers A, Wells MC, Stern M (2007) Phosphatidylinositol 3 kinase and Akt nonautonomously promote perineurial glial growth in *Drosophila* peripheral nerves, *J. Neurosci*, 27: 279-288.

Yager J, Richards S, Hekmat-Scafe D, Hurd D, Sunderesan V, Caprette D, Saxton W, Carlson J, Stern M (2001) Control of *Drosophila* perineurial growth by two interacting neurotransmitter-mediated signaling pathways, *Proc. Natl. Acad. Sci.*, 98: 10445-10450.

CONTACT INFORMATION:

Michael Stern
Dept. of Biochemistry MS-140
Rice University
PO Box 1892
Houston, TX 77251-1892
stern@rice.edu
(713) 348-5351
FAX: (713) 348-5154